REAGENTS AND MATERIALS REQUIRED:

1. Each kit contains 20 test devices, each sealed in a foil pouch with three items inside:
   a. One cassette device.
   b. One plastic dropper
   c. One desiccant.
2. Sample diluant (1 bottle, 3 mL)
3. One package insert (instruction for use).
4. Positive Control (available on request).
5. Negative Control (available on request).
6. Clock or Timer (not provided).

WARNINGS AND PRECAUTIONS:

For in Vitro Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15°C-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use the test device beyond the expiration date printed on the sealed pouch.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens.
8. Do not perform the test in a room with strong air flow, i.e. electric fan or strong air-conditioning.
9. Handle the Negative and Positive Control in the same manner as patient specimens.
10. Dispose of all specimens and materials used to perform the test as biohazardous waste.
11. Disposal of all specimens and materials used to perform the test as biohazardous waste.
12. The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Reading result after 15 minutes may give erroneous results.

INTENDED USE:

The HEV IgM Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of IgM antibody to Hepatitis E virus (HEV) in human serum or plasma. It is intended to be used as a screening test and as an aid in the diagnosis of infection with HEV. Any reactive specimen with the HEV IgM Rapid Test must be confirmed with alternative testing methods and clinical findings.

SUMMARY AND EXPLANATION:

Hepatitis E, a major form of enterically transmitted hepatitis, is widespread in many developing countries but is currently considered an emerging threat to other parts of the world. HEV is a non-enveloped, positive-sense, single-stranded RNA virus (3) which is currently classified within the family Caliciviridae. It is mainly transmitted through faecal-oral route. At least four major genotypes of HEV have been recognized (4, 5) genotypes 1 and 2 are restricted to humans while genotypes 3 and 4 can infect both humans and animals. Antibody response peaks at about one month after initial infection. Arboviral HEV is detected in <80% patient and persists for 3-12 months. Anti-HEV IgM antibody is also a well-established marker of recent infection (6) and most convenient one for diagnosis (7).

Reliable techniques for anti-HEV detection such as immunofluorescence and immune electron microscopy (IEM) have been developed. However, these techniques require labour-intensive procedures that are not available to many laboratories. The HEV IgM Rapid Test is designed to detect anti-HEV IgM in less than 15 minutes by untrained or minimally skilled personnel, without cumbersome laboratory equipment.

PRINCIPLE OF THE TEST:

The HEV IgM Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of:
1. A burgundy coloured conjugate pad containing HEV antigens conjugated with colloidal gold (HEV conjugates) and rabbit IgG gold conjugates.
2. A nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with monoclonal anti-human IgM antibody, and the C line is pre-coated with goat anti-rabbit IgG antibody.

When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the cassette. Anti-HEV IgM if present in the specimen will bind to the HEV conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM, forming a burgundy coloured T line, indicating a HEV IgM positive test result.

Absence of the test line suggests a negative result. The test contains an internal control (C line) which should exhibit a burgundy coloured band of the immunocomplex of goat anti-rabbit IgG and HEV-IgG gold conjugate regardless of the colour development on the T line. Otherwise, the test result is invalid and the specimen must be retested with another device.

When a new test kit is used:

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by vein puncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labelled tube.

When a new test kit is used:

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by vein puncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labelled tube.

EXPECTED RESULTS:

1. NEGATIVE RESULT: If only the C line is developed, the test indicates that no detectable IgM anti-HEV is present in the specimen. The test result is negative or non-reactive.

2. POSITIVE RESULT: If both C and T lines are developed, the test indicates for the presence of IgM anti-HEV in the specimen. The result is positive or reactive.

TEST PROCEDURE:

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen’s ID number.

Step 4: Fill the pipette dropper with the specimen.

Step 5: Set up the timer.

Step 6: Results can be read in 15 minutes. Positive results can be visible as soon as 1 minute. Don’t read result after 15 minutes. To avoid confusion, discard the test device after interpreting the result. Take picture for permanent record if required.

QUALITY CONTROL:

Using individual HEV IgM Rapid Test cassettes as described in the Assay Procedure above, run 1 Positive Control and 1 Negative Control (provided upon request) under the following circumstances to monitor test performance:

1. A new operator uses the kit, prior to performing testing of specimens.
2. A new test kit is used.
3. A new shipment of kits is used.
4. The temperature used during storage of the kit falls outside of 2°C-8°C.
5. The temperature of the test area falls outside of 15°C-30°C.

EXPECTED RESULTS:

1. NEGATIVE RESULT: If only the C line is developed, the test indicates that no detectable IgM anti-HEV is present in the specimen. The test result is negative or non-reactive.

2. POSITIVE RESULT: If both C and T lines are developed, the test indicates for the presence of IgM anti-HEV in the specimen. The result is positive or reactive.
Samples with positive or reactive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. **INVALID**: If no C line is developed, the assay is invalid regardless of colour development on the T line as indicated below. Repeat the assay with a new device.

**PERFORMANCE CHARACTERISTICS:**

**Clinical Performance**

A total of 1060 samples from susceptible subjects were tested by the HEV IgM Rapid Test and by a commercial ELISA test. Comparison for all subjects is shown in the following table:

<table>
<thead>
<tr>
<th>HEV ELISA</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>314</td>
<td>6</td>
<td>320</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>734</td>
<td>740</td>
</tr>
<tr>
<td>Total</td>
<td>320</td>
<td>740</td>
<td>1060</td>
</tr>
</tbody>
</table>

Relative Sensitivity: 98.1%, Relative Specificity: 99.2%, Overall Agreement: 98.9%

**LIMITATIONS:**

1. The assay procedure and the assay result interpretation must be followed closely when testing the presence of anti-HEV IgM in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The HEV IgM Rapid Test is limited to the qualitative detection of anti-HEV IgM in human serum or plasma. The intensity of the test band does not have linear correlation with the antibody titer in the specimen.
3. A negative or non-reactive result for an individual subject indicates absence of detectable anti-HEV IgM. However, a negative or non-reactive test result does not preclude the possibility of exposure to or infection with HEV.
4. A negative or non-reactive result can occur if the quantity of the anti-HEV IgM present in the specimen is below the detection limits of the assay, or if the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. If the symptom persists, while the result from HEV IgM Rapid Test is negative or non-reactive result, it is recommended to re-sample the patient few days late or test with an alternative test method such as ELISA or PCR.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

**BIBLIOGRAPHY:**


**SYMBOLS:**

The following symbols are used in the labelling of Audit Diagnostics systems:

1. Consult instructions for use
2. For in vitro diagnostic use only
3. Authorized Representative
4. Authorized Representative
5. Store between 2-30°C
6. Do not reuse
7. Tests per kit
8. Use by

**Manufactured By:**

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